Important Role of Angiotensin II–Mediated c-Jun NH_2-Terminal Kinase Activation in Cardiac Hypertrophy in Hypertensive Rats

Yasukatsu Izumi, Shokei Kim, Yumei Zhan, Masashi Namba, Hideo Yasumoto, Hiroshi Iwao

Abstract—In vitro studies on the role of the mitogen-activated protein (MAP) kinase family (extracellular signal-regulated kinase [ERK], c-Jun NH_2-terminal kinase [JNK], and p38) in cardiac hypertrophic response have produced confusing and contradictory results. We examined the in vivo role of the angiotensin II type 1 (AT_1) receptor in cardiac MAP kinase activities during both the onset and development of cardiac hypertrophy in stroke-prone spontaneously hypertensive rats (SHRSP). In both the acute and chronic phases of cardiac hypertrophy in SHRSP, cardiac JNK activities were significantly increased compared with those in normotensive rats, whereas there was no prominent increase in cardiac ERK or p38 activities in SHRSP. Losartan, an AT_1 receptor antagonist, prevented the onset of cardiac hypertrophy and regressed the progression of cardiac hypertrophy in SHRSP, being accompanied by the reduction of JNK activity and activator protein-1 (AP-1) activity in SHRSP. However, in spite of the normalization of blood pressure, hydralazine did not prevent or regress cardiac hypertrophy and did not decrease JNK or AP-1 activity in SHRSP. Inversely, hydralazine significantly increased the cardiac ERK activity in SHRSP by enhancing its phosphorylation. In conclusion, we have obtained the first evidence that the AT_1 receptor is involved in the enhanced cardiac JNK activity in both the onset and development of cardiac hypertrophy of hypertensive rats. We propose that JNK is involved in AT_1 receptor–mediated cardiac hypertrophy in vivo, in part mediated by the activation of AP-1. (***Hypertension.* 2000;36:511-516.**)

Key Words: protein kinases ■ hypertrophy ■ hypertension, experimental ■ angiotensin ■ rats

Accumulating in vivo evidence indicates that angiotensin II (Ang II), via the AT_1 receptor, plays a central role in the onset and development of cardiac hypertrophy and remodeling.[1,2] However, the investigations on molecular mechanism of Ang II–mediated cardiac hypertrophy are almost limited to in vitro studies on cultured cardiac myocytes (mostly neonatal cardiac myocytes), and the in vivo mechanism of Ang II–mediated pathological cardiac hypertrophy remains poorly understood.

Extracellular signal-regulated kinase (ERK), c-Jun NH_2-terminal kinase (JNK), and p38 mitogen-activated protein (MAP) kinase are protein serine/threonine kinases that belong to MAP kinase family.[3–5] These 3 MAP kinases are proposed to play a key role not only in the activation of various transcription factors, such as activator protein-1 (AP-1), and the regulation of various gene expressions but also in the hypertrophic response in cultured cardiac myocytes.[6–9] Ang II has been reported to activate ERK[10] and JNK[11] in cultured cardiac myocytes. However, despite the detailed investigations on the regulation and role of MAP kinases in cultured cardiac myocytes, the in vivo regulation and role of MAP kinases in cardiac hypertrophy are poorly understood.

Recently, we have determined the activities of cardiac ERK and JNK in stroke-prone spontaneously hypertensive rats (SHRSP) from the phase of prehypertension to established cardiac hypertrophy, and we have found that cardiac JNK activity is significantly increased throughout the hypertensive phase in SHRSP compared with normotensive Wistar-Kyoto rats (WKY), whereas cardiac ERK activity is only slightly increased.[12] In the present study, to elucidate the involvement of the AT_1 receptor in MAP kinase activities in the cardiac hypertrophy of SHRSP, we compared the effects of losartan, an AT_1 receptor antagonist, and hydralazine on cardiac ERK and JNK activities and AP-1 DNA binding activity in SHRSP. We also examined cardiac p38 MAP kinase in SHRSP. We have obtained the first evidence indicating that the AT_1 receptor is involved in the enhanced JNK activity during both the acute and chronic phases of cardiac hypertrophy in hypertensive rats, and we suggest that JNK may participate in AT_1 receptor–mediated cardiac hypertrophy in vivo.

Methods

Experimental Protocol

All procedures were in accordance with institutional guidelines for animal research. Male SHRSP[13] and WKY, which were purchased

Received February 4, 2000; first decision February 29, 2000; revision accepted April 17, 2000.

From the Department of Pharmacology, Osaka City University Medical School, Osaka 545-8585, Japan.

Correspondence to Shokei Kim, MD, Department of Pharmacology, Osaka City University Medical School, 1-4-3 Asahimachi, Abeno, Osaka 545-8585, Japan. E-mail kims@med.osaka-cu.ac.jp

Hypertension is available at http://www.hypertensionaha.org

from Japan SLC (Shizuoka, Japan), were fed standard laboratory chow (CE-2, Clea Japan) and given tap water ad libitum. Losartan, an AT_1 receptor antagonist, was a gift from MERCK Research Laboratories (Rahway, New Jersey). Hydralazine was purchased from WAKO Pure Chemical Industries, Ltd.

The first series of experiments was performed to examine the role of MAP kinases in the onset of cardiac hypertrophy. Preliminary experiments showed no cardiac hypertrophy in 6-week-old SHRSP compared with age-matched WKY rats, as shown by no differences in left ventricular (LV) weight. On the other hand, compared with age-matched WKY, 8-week-old SHRSP had an elevated LV weight, indicating that LV hypertrophy had begun to occur at 6 to 8 weeks of age. Therefore, to examine the possible involvement of MAP kinases in the acute phase of cardiac hypertrophy, 6-week-old SHRSP were treated with (1) vehicle (0.5% carboxymethyl cellulose) (n=7), (2) 20 mg · kg^{-1} · d^{-1} losartan (n=7), or (3) 10 mg · kg^{-1} · d^{-1} hydralazine (n=7) for 2 weeks. Losartan, suspended in 0.5% carboxymethyl cellulose, and vehicle were given orally to rats by gastric gavage every morning once a day for 14 days (from the age of 6 to 8 weeks). Hydralazine was dissolved in distilled water and given to rats as drinking water for the same period.

A second series of experiments was undertaken to examine the role of MAP kinases in the chronic phase of LV hypertrophy. Twenty-week-old SHRSP were used because this age of SHRSP had LV hypertrophy and remodeling that were already established. SHRSP were separated into 4 groups and were treated with (1) vehicle (0.5% carboxymethyl cellulose) (n=8), (2) 30 mg · kg^{-1} · d^{-1} losartan (n=7), (3) 60 mg · kg^{-1} · d^{-1} losartan (n=7), or (4) 50 mg · kg^{-1} · d^{-1} hydralazine (n=7) from 20 to 22 weeks of age, in the same manner as in the first experiments.

Blood pressure and heart rate were periodically measured by the tail-cuff method with a sphygmomanometer (TK-370A, Unicom Inc). After drug treatment, the rats were killed by decapitation, and the whole heart was rapidly excised and rinsed with cold saline. The left ventricle was then separated from the right ventricle and atria, immediately frozen in liquid nitrogen, and stored at −80°C until protein extraction for MAP kinase assay or Northern blot analysis.

Preparation of Cardiac Protein Extracts

For MAP kinase assay, cardiac protein extracts were prepared, as described in detail in our previous reports.[12,14]

Measurement of Cardiac ERK Activity

The assay of ERK activities was performed by using an in-gel kinase method with myelin basic protein as the substrate, as described in detail in our previous reports.[12,14,15] The density of autoradiograms was analyzed with a bioimaging analyzer (BAS-2000, Fuji Photo Film Co).

Measurement of Cardiac JNK Activity

The assay of JNK activities was also performed by using an in-gel kinase method, as described in detail in our previous reports.[12,14,15] We used glutathione-S-transferase–c-Jun (1-79) protein as JNK substrate.[16] Autoradiographic bands were also analyzed with the bioimaging analyzer (BAS-2000, Fuji Photo Film Co).

Western Blot Analysis

Cardiac ERK and p38 MAP kinase protein levels were measured with Western blot analysis, as published in detail by us.[15] Antibodies used were as follows: phospho-specific ERK polyclonal antibody (Promega), anti–total ERK antibody (Santa Cruz), anti–phospho-p38 antibody (New England Biolabs, Inc), and anti–total p38 antibody (Santa Cruz). The intensity of the bands was measured with an optical scanner (EPSON GT-8000, Seoko) by use of the public domain NIH Image program.

Northern Blot Analysis

All procedures were performed as described in detail in our previous reports.[17] The densities of an individual mRNA band were measured with use of the bioimaging analyzer (BAS-2000, Fuji Photo Film Co).

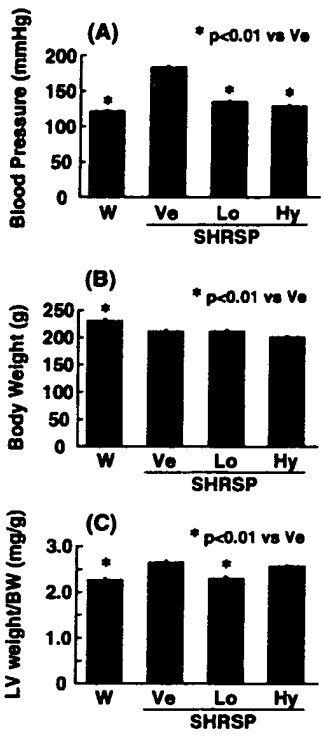

Figure 1. Blood pressure (A), body weight (B) and ratio of LV weight to body weight (LV weight/BW) (C) for 8-week-old WKY (W) and SHRSP treated with vehicle (Ve), losartan (Lo), and hydralazine (Hy) for 2 weeks (from 6 to 8 weeks of age). Each bar represents mean±SEM (n=7).

Preparation of Cardiac Nuclear Extracts

For the gel mobility shift assay, nuclear protein extracts were prepared as described in our previous reports.[14]

Gel Mobility Shift Assay

A gel mobility shift assay of AP-1 DNA binding activity was performed as described in our previous report.[14,15,18] The sequence of the AP-1 consensus oligonucleotide probe, which was used for the gel mobility shift assays, was 5′-CGCTTGA*TGACTCA*GCCGGAA-3′.[19] A supershift assay was carried out by using rabbit polyclonal IgG against c-Fos or c-Jun (Santa Cruz Biotechnology, Inc) as described.[14]

Statistical Analysis

All data are presented as mean±SEM. Statistical significance between 2 groups was determined with an unpaired Student *t* test. For comparison among >2 groups, statistical significance was determined with 1-way ANOVA and the Duncan multiple range test. Differences were considered statistically significant at a value of $P<0.05$.

Results

Effects of Losartan and Hydralazine on Cardiac MAP Kinases During Acute Phase of Cardiac Hypertrophy

As shown in Figure 1A, after 13 days of treatment, blood pressure of SHRSP (183±3 mm Hg) was significantly decreased by either losartan (135±2 mm Hg) or hydralazine(129±3 mm Hg). We also measured blood pressures for each group of rats at 7 days after the start of drug treatment, and similar results were obtained (data not shown). As shown in Figure 1B, there was no significant difference in body

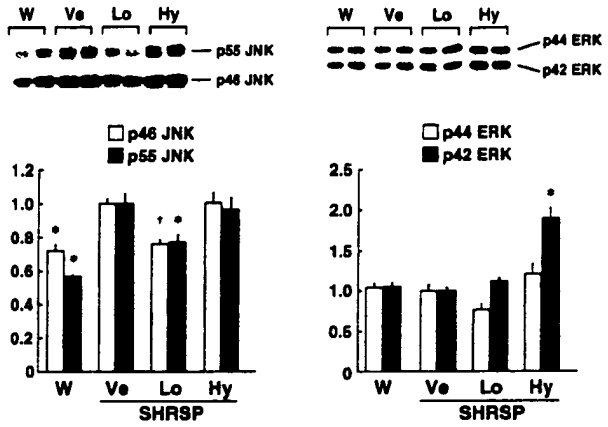

Figure 2. Effects of Lo and Hy on LV JNK and ERK activities in SHRSP during the onset of cardiac hypertrophy. MAP kinase activities were determined by in-gel kinase assay. Each bar represents mean±SEM (n=7). *P<0.01 vs Ve-treated SHRSP.

weight among the 3 SHRSP groups treated with vehicle, losartan, and hydralazine. Despite similar hypotensive effects between losartan and hydralazine, the LV weight of 8-week-old SHRSP (2.65±0.03 mg/g body wt), which was greater than that of WKY (2.27±0.03 mg/g body wt), was significantly reduced by losartan (2.30±0.04 mg/g body wt, P<0.01) but not by hydralazine (2.55±0.05 mg/g body wt) (Figure 1C). The failure of suppression of cardiac hypertrophy by hydralazine was consistent with a previous report.[20]

As shown by the in-gel kinase assay in Figure 2, LV JNK consisted of 2 isoforms, p46JNK and p55JNK. LV JNK activities of 8-week-old SHRSP, which were significantly higher than the activities of WKY, were significantly decreased by losartan but not by hydralazine. On the other hand, LV ERK activities of SHRSP, which consisted of p44ERK and p42ERK, were unaffected by losartan but significantly increased by hydralazine.

Effects of Losartan and Hydralazine on Blood Pressure, Heart Rate, and LV Weight During Chronic Phase of Cardiac Hypertrophy

As shown in Figure 3A, after 13 days of treatment, the blood pressure of SHRSP treated with 30 and 60 mg/kg losartan (153±3 and 135±2 mm Hg, respectively) or hydralazine (121±3 mm Hg) was similar to that of WKY rats. As shown in Figure 3, the heart rate of vehicle-treated SHRSP (352±6 bpm) was significantly higher than that of the age-matched WKY (308±4 bpm, P<0.01). Treatment of SHRSP with losartan did not alter the heart rate of SHRSP. However, hydralazine significantly increased the heart rate of SHRSP (P<0.01). We also measured blood pressures and heart rates for each group of rats at 7 days after the start of drug treatment, and similar results were obtained (data not shown).

As shown in Figure 3C, the LV weight of vehicle-treated SHRSP was significantly greater than that of age-matched WKY (3.50±0.06 versus 2.34±0.06 mg/g, P<0.01). Treatment with losartan significantly reduced the LV weight in SHRSP. On the other hand, in spite of the normalization of blood pressure, hydralazine failed to decrease the LV weight in SHRSP.

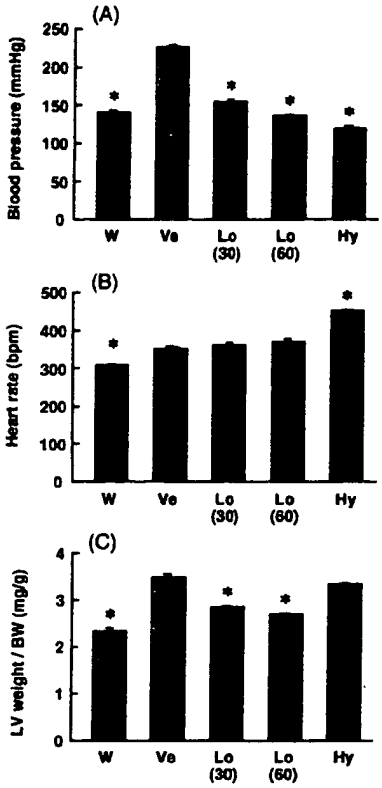

Figure 3. Blood pressure (A), heart rate (B), and LV weight/BW ratio (C) for 22-week-old WKY (W) and SHRSP treated with Ve, Lo at 30 or 60 mg/kg, and Hy. WKY were treated with vehicle for the same period. Each bar represents mean±SEM (n=7 or 8). *P<0.01 vs Ve-treated SHRSP.

Effects of Losartan and Hydralazine on Cardiac JNK and ERK Activities During Chronic Phase of Cardiac Hypertrophy

As shown by in-gel kinase assay in Figure 4A, LV p46JNK and p55JNK activities in vehicle-treated SHRSP were 1.8- and 1.9-fold higher, respectively, than those in WKY (P<0.01), being consistent with our previous report.[12] Losartan decreased the LV p46JNK and p55JNK activities of SHRSP. On the other hand, in spite of the normalization of blood pressure, hydralazine did not change LV p46JNK or p55JNK activity in SHRSP.

Effects of Losartan and Hydralazine on Cardiac ERK Activities During Chronic Phase of Cardiac Hypertrophy

As shown by in-gel kinase assay in Figure 4B, LV p44ERK and p42ERK activity tended to be slightly higher in SHRSP than in WKY, being consistent with our previous report.[12] Losartan did not change LV p44ERK or p42ERK activity in SHRSP. In contrast, hydralazine treatment increased LV p44ERK and p42ERK activity in SHRSP by 1.9- and 2.3-fold (P<0.01), respectively.

As shown by Western blot analysis in Figure 5, losartan did not significantly alter LV immunoreactive phospho-ERK levels, whereas hydralazine significantly increased phospho-p44ERK and phospho-p42ERK levels by 1.9- and 3.0-fold, respectively. However, total p44ERK or p42ERK protein levels were not changed by losartan or hydralazine.

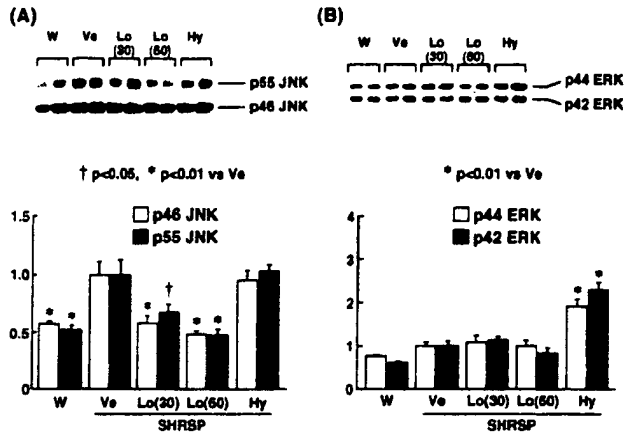

Figure 4. Effects of Lo and Hy on LV JNK (A) and ERK (B) activities of SHRSP. Top panels are representative autoradiograms of LV JNK and ERK activities for each group obtained by in-gel kinase assay. Bottom panels show LV JNK and ERK activities for each group. Each bar represents mean±SEM (n=7 or 8). In both p46JNK and p55JNK activity or in both p44ERK and p42ERK activity, the mean value of JNK or ERK activity in Ve-treated SHRSP is represented as 1.

Effects of Losartan and Hydralazine on Cardiac AP-1 DNA Binding Activity

As shown by gel mobility shift assay in Figure 6A, 2 bands were detected by the incubation of LV nuclear extracts from 22-week-old SHRSP with radiolabeled AP-1 consensus oligonucleotide probe. Addition of increasing concentrations of unlabeled AP-1 consensus oligonucleotide effectively competed for the slower migrating band but not for the faster migrating band, indicating that the slower migrating band represented specific AP-1 DNA binding, whereas the faster migrating band represented nonspecific binding. Furthermore, this slower migrating band was supershifted by the addition of anti–c-Jun or anti–c-Fos antibody, whereas the faster migrating band was not affected by these antibodies. These observations show that the slower migrating band represented specific AP-1 complexes containing c-Fos and c-Jun.

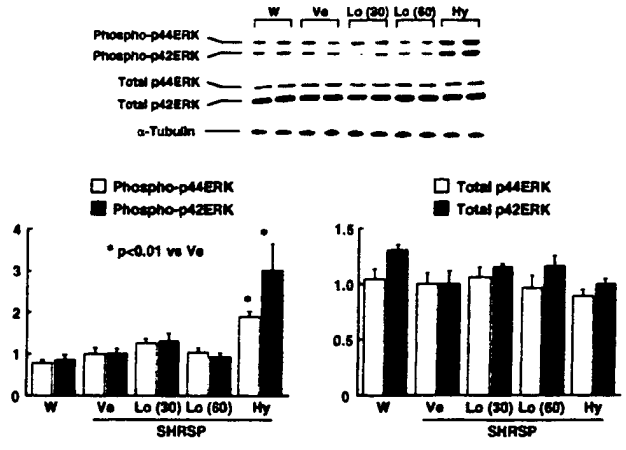

Figure 5. Effects of Lo and Hy on LV phospho-ERK and total ERK protein levels of SHRSP. Top panel shows representative autoradiograms obtained by Western blot analysis. Each bar represents mean±SEM (n=7 or 8). The mean value in Ve-treated SHRSP is represented as 1.

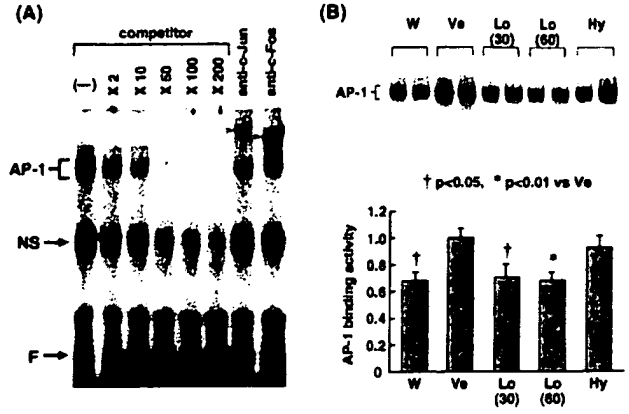

Figure 6. Effects of Lo and Hy on LV AP-1 DNA binding activity of SHRSP. A, A sample of LV nuclear extracts from Ve-treated SHRSP was incubated with ^{32}P-labeled AP-1 consensus oligonucleotide probe in the absence of unlabeled AP-1 oligonucleotide (–) and in the presence of 2-, 10-, 50-, 100-, and 200-fold molar excess of unlabeled AP-1 competitor (×2, ×10, ×50, ×100, and ×200, respectively). Furthermore, supershift assay was carried out with anti–c-Jun) and anti–c-Fos antibody. Supershifted bands are shown by arrows. Half bracket indicates specific AP-1 binding complexes. NS indicates nonspecific binding; F, free probe. B. Top panel shows representative autoradiograms of LV AP-1 binding activity for each group. Bottom panel shows LV AP-1 binding activity for each group. Each bar represents mean±SEM (n=7 or 8). The mean value of AP-1 binding activity in vehicle-treated SHRSP is represented as 1.

As shown in Figure 6B, LV AP-1 DNA binding activity in vehicle-treated SHRSP was 1.5-fold higher than that in WKY ($P<0.05$). Losartan at 30 or 60 mg/kg significantly decreased LV AP-1 binding activity in SHRSP. On the other hand, hydralazine did not reduce LV AP-1 binding activity.

p38 MAP Kinase During Acute and Chronic Phases of Cardiac Hypertrophy

As shown by Northern blot analysis and Western blot analysis in Figure 7, there was no significant difference in LV p38 mRNA levels, phospho-p38 (active p38) levels, and total p38 protein levels between WKY and SHRSP at the age of 8 and 22 weeks (acute and chronic phase, respectively, of cardiac hypertrophy).

Discussion

Although studies of the significance of the MAP kinase family (including ERK, JNK, and p38) in cultured cardiac myocytes in vitro have been extensively performed, they have produced confusing and contradictory results.[6,8] Furthermore, little information is available on the regulation of the MAP kinase family in in vivo cardiac hypertrophy. In our present in vivo study, we obtained the first evidence indicating that the activation of JNK is more prominent than ERK and p38 activation during both the acute and chronic phases of in vivo cardiac hypertrophy and that there is a significant difference among regulatory mechanism of these 3 MAP kinases. Furthermore, we found that Ang II is directly involved in JNK activation during either the onset or development of cardiac hypertrophy. Taken together with recent in vivo findings demonstrating that inhibition of JNK by gene transfer of the dominant inhibitory mutant of SEK-1 (the imme-

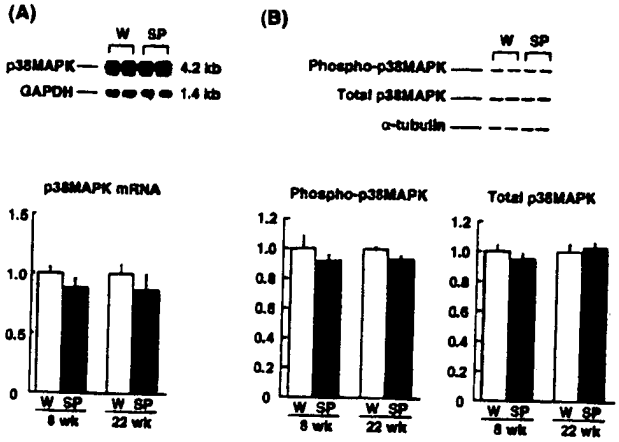

Figure 7. LV p38 MAP kinase in 8- and 22-week-old WKY (W) and SHRSP (SP). A, LV p38 MAP kinase mRNA levels were determined by Northern blot analysis and corrected for GAPDH mRNA levels. B, Phospho-p38 MAP kinase (active p38) and total p38 protein levels were determined by Western blot analysis with their specific antibodies and corrected for α-tubulin. Each bar represents mean±SEM (n=7).

diate upstream activator of JNK) inhibits pressure overload–induced rat cardiac hypertrophy,[21] our observations support the notion that JNK participates in AT_1 receptor–mediated cardiac hypertrophy in vivo.

SHRSP is regarded as a useful animal model for investigation of the mechanism of pathological cardiac hypertrophy and remodeling in vivo. We have previously reported that SHRSP develop not only prominent LV hypertrophy and fibrosis but also an enhanced expression of cardiac remodeling–associated genes, such as transforming growth factor-β1 and collagen mRNA.[22,23] These pathological changes were at least in part mediated by AT_1 receptors.[22] Furthermore, more recently, the use of in-gel kinase assay has allowed us to successfully measure ERK and JNK activities in cardiac tissues.[12,14] By using in-gel kinase assays, we have previously found that LV JNK activity is significantly increased in SHRSP compared with WKY and that LV ERK is slightly increased in SHRSP, thereby suggesting that JNK and ERK may play some role in LV hypertrophy and remodeling in SHRSP.[12] In the present study, we first determined cardiac p38 MAP kinase at acute and chronic phases of cardiac hypertrophy in SHRSP (Figure 7) and obtained no evidence supporting the involvement of p38 MAP kinase in the acute or chronic phases of cardiac hypertrophy. All these findings led us to examine the role of the AT_1 receptor in cardiac JNK and ERK activities during the acute and chronic phases of cardiac hypertrophy of SHRSP.

In the present experiments on the acute phase of cardiac hypertrophy, losartan prevented the onset of cardiac hypertrophy, indicating that the AT_1 receptor is involved in the onset of cardiac hypertrophy. Furthermore, the prevention of cardiac hypertrophy by losartan was associated with the decrease in LV JNK activities. Also, in the chronic phase of cardiac hypertrophy, losartan treatment regressed LV hypertrophy in SHRSP, accompanied by the reduction of cardiac JNK activities. On the other hand, hydralazine treatment did not prevent or regress LV hypertrophy and did not decrease JNK activity in SHRSP, showing that the increased JNK activity in SHRSP was not solely due to high blood pressure. These observations, taken together with our recent findings that in vivo Ang II infusion in rats increases cardiac JNK activity preceding the onset of cardiac hypertrophy,[14] indicate that the AT_1 receptor itself contributes to the enhanced activities of these 2 JNK isoforms in SHRSP during both the acute and chronic phases of cardiac hypertrophy.

JNK is the major kinase enhancing c-Jun transactivational activity by its phosphorylation.[24] Furthermore, JNK promotes c-*fos* gene expression. c-Jun and c-Fos proteins form the transcriptional factor AP-1 complex and stimulate various gene expressions by binding the AP-1 consensus sequence present in their promoter region.[24] Interestingly, various cardiac hypertrophy–related genes, such as transforming growth factor-β1, collagen, and skeletal α-actin, whose expressions are enhanced in SHRSP,[22,23] have the AP-1 consensus sequence in their promoter regions.[8,24] Previously, we have reported that losartan significantly inhibits the above-mentioned cardiac hypertrophy–related gene expressions in the LV of SHRSP, which are at least in part mediated by direct inhibition of the cardiac AT_1 receptor independent of its hypotensive effect.[22] Furthermore, very recently, in another study, we have found that the activation of cardiac JNK in rats by Ang II infusion, without ERK activation, is followed by a significant increase in cardiac AP-1 activity.[14] These findings led us to examine the effect of losartan on LV AP-1 DNA binding activity in SHRSP. In the present study, we obtained the first evidence indicating that LV AP-1 binding activity, which contained c-Fos and c-Jun proteins, was significantly increased in SHRSP compared with WKY. Notably, losartan, but not hydralazine, significantly decreased LV AP-1 DNA binding activity in SHRSP. These findings suggest that JNK, partially mediated by AP-1, might participate in the cardiac hypertrophic response of SHRSP. However, further study is needed to elucidate whether the increase in JNK activity in SHRSP was due to cardiomyocytes, fibroblasts, or both.

In the present study, in contrast to JNK, LV ERK activity in SHRSP was not associated with the prevention or regression of LV hypertrophy by AT_1 receptor antagonists. Notably, in both the acute and chronic phase of cardiac hypertrophy, hydralazine treatment significantly increased LV ERK activity in SHRSP. It is possible that the increase in ERK activity is due to the enhanced phosphorylation of ERK. Alternatively, the increase in ERK protein levels may participate in the increase in ERK activity. To elucidate these possibilities, we carried out Western blot analysis (Figure 5) and found that the elevated ERK activity by hydralazine was due to the enhanced phosphorylation of ERK but not the increase in total ERK protein expression. The present study did not permit us to elucidate the mechanism and significance of the elevated ERK activity by hydralazine. However, in the present study, hydralazine significantly increased the heart rat of SHRSP, indicating activation of the sympathetic nervous system. In cultured neonatal rat cardiac myocytes, ERK is shown to be activated by either α- or β-adrenergic agonists.[25] Therefore, the elevation of cardiac ERK activity by hydralazine may be mediated by the activation of the sympathetic

nervous system, and the elevation of ERK activity may in part explain the failure of prevention or regression of cardiac hypertrophy by hydralazine despite it hypotensive effect. However, further study is needed to demonstrate our assumption.

The present study provided no evidence for the participation of ERK in cardiac hypertrophy in SHRSP. However, the activation of ERK has been reported to be involved in cardiac myocyte hypertrophy and in the gene expression of atrial natriuretic factor, the fetal gene reexpressed during cardiac hypertrophy.[9,26] Therefore, it cannot be ruled out that ERK may play some role in the pathophysiology of cardiac hypertrophy in vivo.

In conclusion, we have obtained the first evidence indicating that the AT_1 receptor is involved in the enhanced cardiac JNK activity in both the onset and development of cardiac hypertrophy in hypertensive rats. Taken together with recent in vivo evidence that the inhibition of cardiac JNK activation by gene transfer of the dominant inhibitory mutant of SEK-1 prevents pressure overload–induced rat cardiac hypertrophy,[21] our present study supports the notion that JNK is involved in AT_1 receptor–mediated cardiac hypertrophy in vivo. Further detailed in vivo study of the role of JNK in cardiac hypertrophy seems to provide new insight into the molecular mechanism of Ang II–mediated cardiac hypertrophy and remodeling in vivo.

Acknowledgments

This work was supported in part by a Grant-in-Aid for Scientific Research (09470527 and 09670101) from the Ministry of Education, Science, Sports, and Culture of Japan.

References

1. Timmermans PBMWM, Wong PC, Chiu AT, Herblin WF, Benfield P, Carini DJ, Lee RJ, Wexler R, Saye J, Smith R. Angiotensin II receptors and angiotensin II receptor antagonists. *Pharmacol Rev.* 1993;45: 205–251.
2. Kim S, Iwao H. Molecular and cellular mechanisms of angiotensin II-mediated cardiovascular and renal diseases. *Pharmacol Rev.* 2000;52: 11–34.
3. Davis RJ. MAPKs: new JNK expands the group. *Trends Biochem Sci.* 1994;19:470–473.
4. Kyriakis JM, Avruch J. Sounding the alarm: protein kinase cascades activated by stress and inflammation. *J Biol Chem.* 1996;271: 24313–24316.
5. Nishida E, Gotoh Y. The MAP kinase cascade is essential for diverse signal transduction pathways. *Trends Biochem Sci.* 1993;18:128–131.
6. Sugden PH, Clerk A. 'Stress-responsive' mitogen-activated protein kinases (c-Jun N-terminal kinases and p38 mitogen-activated protein kinases) in the myocardium. *Circ Res.* 1998;83:345–352.
7. Sadoshima J, Izumo S. Mechanical stretch rapidly activates multiple signal transduction pathways in cardiac myocytes: potential involvement of an autocrine/paracrine mechanism. *EMBO J.* 1993;12:1681–1692.
8. Force T, Pombo CM, Avruch JA, Bonventre JV, Kyriakis JM. Stress-activated protein kinases in cardiovascular disease. *Circ Res.* 1996;78: 947–953.
9. Glennon PE, Kaddoura S, Sale EM, Sale GJ, Fuller SJ, Sugden PH. Depletion of mitogen-activated protein kinase using an antisense oligodeoxynucleotide approach downregulates the phenylephrine-induced hypertrophic response in rat cardiac myocytes. *Circ Res.* 1996;78:954–961.
10. Yamazaki T, Komuro I, Kudoh S, Zou Y, Shiojima I, Mizuno T, Takano H, Hiroi Y, Ueki K, Tobe K. Angiotensin II partly mediates mechanical stress–induced cardiac hypertrophy. *Circ Res.* 1995;77:258–265.
11. Kudo S, Komuro I, Mizuno T, Yamazaki T, Zou Y, Shiojima I, Takekoshi N, Yazaki Y. Angiotensin II stimulates c-Jun NH_2-terminal kinase in cultured cardiac myocytes of neonatal rats. *Circ Res.* 1997;80:139–146.
12. Izumi Y, Kim S, Murakami T, Yamanaka S, Iwao H. Cardiac mitogen-activated protein kinase activities are chronically increased in stroke-prone hypertensive rats. *Hypertension.* 1998;31:50–56.
13. Okamoto K, Yamori Y, Nagaoka A. Establishment of the stroke-prone spontaneously hypertensive rat(SHR). *Circ Res.* 1974;35(suppl I):I-143–I-153.
14. Yano M, Kim S, Izumi Y, Yamanaka S, Iwao H. Differential activation of cardiac c-Jun amino-terminal kinase and extracellular signal-regulated kinase in angiotensin II–mediated hypertension. *Circ Res.* 1998;83: 752–760.
15. Kim S, Izumi Y, Yano M, Hamaguchi A, Miura K, Yamanaka S, Miyazaki H, Iwao H. Angiotensin blockade inhibits activation of mitogen-activated protein kinases in rat balloon-injured artery. *Circulation.* 1998;97:1731–1737.
16. Hibi M, Lin A, Smeal T, Minden A, Karin M. Identification of an oncoprotein- and UV-responsive protein kinase that binds and potentiates the c-Jun activation domain. *Genes Dev.* 1993;7:2135–2148.
17. Ohta K, Kim S, Wanibuchi H, Ganten D, Iwao H. Contribution of local renin-angiotensin system to cardiac hypertrophy, phenotypic modulation, and remodeling in TGR(mRen2)27 transgenic rats. *Circulation.* 1996;94: 785–791.
18. Hamaguchi A, Kim S, Yano M, Yamanaka S, Iwao H. Activation of glomerular mitogen-activated protein kinases in angiotensin II-mediated hypertension. *J Am Soc Nephrol.* 1998;9:372–380.
19. Lee W, Mitchell P, Tjian R. Purified transcription factor AP-1 interacts with TPA-inducible enhancer elements. *Cell.* 1987;49:741–752.
20. Leenen FH, Harmsen E. Antihypertensive drugs and cardiac trophic mechanisms. *J Cardiovasc Pharmacol.* 1991;17:S50–S7.
21. Choukroun G, Hajjar R, Fry S, del Monte F, Haq S, Guerrero JL, Picard M, Rosenzweig A, Force T. Regulation of cardiac hypertrophy in vivo by the stress-activated protein kinases/c-Jun NH(2)-terminal kinases. *J Clin Invest.* 1999;104:391–398.
22. Kim S, Ohta K, Hamaguchi A, Yukimura T, Miura K, Iwao H. Effects of an AT1 receptor antagonist, an ACE inhibitor and a calcium channel antagonist on cardiac gene expressions in hypertensive rats. *Br J Pharmacol.* 1996;118:549–556.
23. Kim S, Ohta K, Hamaguchi A, Omura T, Yukimura T, Miura K, Inada Y, Ishimura Y, Chatani F, Iwao H. Angiotensin II type 1 receptor antagonist inhibits the gene expression of transforming growth factor-b1 and extracellular matrix in cardiac and vascular tissues of hypertensive rats. *J Pharmacol Exp Ther.* 1995;273:509–515.
24. Karin M. The regulation of AP-1 activity by mitogen-activated protein kinases. *J Biol Chem.* 1995;270:16483–16486.
25. Yamazaki T, Kommuro I, Zou Y, Kudoh S, Shiojima I, Hiroi Y, Mizuno T, Aikawa R, Takeno H, Yazaki Y. Norepinephrine induces the raf-1 kinase/mitogen-activated protein kinase cascade through both α1- and β-adrenoceptors. *Circulation.* 1997;95:1260–1268.
26. Thorburn J, Frost JA, Thorburn A. Mitogen-activated protein kinases mediate changes in gene expression, but not cytoskeletal organization associated with cardiac muscle cell hypertrophy. *J Cell Biol.* 1994;126: 1565–1572.